IN VITRO PROPAGATION OF *HEBE SPECIOSA* ON AN ORGANIC - FREE MEDIUM

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ABSTRACT

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Axenic plantlets of *Hebe speciosa* (A. Cunn.) Ckn et Allan (Scrophulariaceae), a garden plant having a pleasant habit with large red/purple inflorescences, have been successfully established in culture. When 'micro-cuttings' consisting of the shoot meristem plus multiple layers of immature leaves were cultured on media containing GA_3, shoot growth was promoted and the emerged shoots with several nodes were rooted on a medium containing IBA. A micropropagation procedure based on plantlet regeneration from single nodal explants of the axenic plantlets was then developed. A culture medium consisting of only 1/10 strength of inorganic MS salts was found to be adequate for shoot development and growth.

KEYWORDS: Gibberellic acid - *Hebe* - micropropagation - Scrophulariaceae.

Abbreviations: BA, benzyladenine; GA3, gibberellic acid; IBA, indole-butyric acid.

INTRODUCTION

Hebe speciosa (A. Cunn.) Ckn et Allan (Scrophulariaceae) is a native New Zealand shrub which is evergreen, reasonably hardy, and has a pleasant habit with large red/purple inflorescenes (Evans 1987). It has great potential to become a popular garden plant in Europe (Brooker *et al* 1988). *H. speciosa* is also recorded as an endangered species (Given 1976) and a simple micropropagation method could therefore be useful in maintaining natural populations. Conventional propagation of *H. speciosa* involves rooting of rather bulky stem cuttings with several nodes, and could meet with varying degrees of success depending on the time of year. Here we report on our investigations into the *in vitro* requirements for the propagation of *H. speciosa*.

MATERIALS AND METHODS

The explants for *in vitro* culture were excised from growing tips of side shoots of mature *Hebe speciosa* plants grown several years in the campus of the University of Canterbury, Christchurch, New Zealand. Each explant consisted of the shoot apex enclosed in multiple layers of immature leaves and the first pair of open leaves, and is termed here as a 'micro-cutting' (approximately 1 to 1.5 cm long).

The micro-cuttings were surface-sterilized in the following sequence of steps:

(1) soaking in sterile water for 15 minutes.

(2) immersing in 70% (v/v) ethanol for 30 seconds.

(3) rinsing briefly in sterile water.

(4) soaking in 100 ml of 2% sodium hypochlorite solution (diluted from a commercial bleach containing 5%, v/v, NaOC l) with 2 drops of Tween 20 for 20 minutes.

(5) rinsing three times with sterile water.

The micro-cuttings were then blotted dry with sterile filter paper and the two open leaves were removed with the cut surface trimmed to within 2 mm of the exposed node before the explants were placed in 400 ml plastic culture vessels each con-

taining 50 mls of media. The cultures were maintained in a room at 25°C under continuous lighting (160 μE $m^{-2}sec^{-1}$).

The basal culture medium, unless otherwise stated, consisted of MS salts (Murashige & Skoog 1962), organic supplements (Nitsch & Nitsch 1969), 3% (w/v) sucrose, and 0.8% (w/v) agar (Davis). The pH of the media was adjusted to 5.8 before autoclaving for 14 mins at 137 kPa. All growth regulators were added prior to autoclaving except GA_3 which was filter sterilized and added after autoclaving.

RESULTS

Micro-cuttings obtained from *H. speciosa* plants grown outdoors during late autumn and winter were first placed on the basal culture medium without addition of any exogenous plant growth regulators. The explants became darkened within five weeks and failed to develop further. Addition of kinetin, BA (both tested at concentrations of 0, 0.1, 1 or 5 mg l^{-1}) and IBA (0, 0.05, 0.1 or 1 mg l^{-1}), either singly or in combination, to the basal culture medium was also largely ineffective in bringing about the development of any new shoots or roots. The only exception was that a very low frequency (about 15%) of the micro-cuttings formed adventitous roots in response to IBA (0.1 mg l^{-1}). Root establishment was followed by shoot development and internodal elongation over a four month period. It is also worth noting that the micro-cuttings cultured on the basal medium containing a cytokinin, either BA or kinetin, initially showed slight swelling of the shoot tip and remained green for up to two months longer than those cultured on the basal medium only.

During the first few days of culture considerable amounts of brown/black pigments were always formed at the cut surface of all micro-cuttings in contact with the basal culture medium. These pigments appeared to have adverse effects on the survival of the micro-cuttings. Addition of ascorbic acid (100 mg l^{-1}) to the basal culture medium had little or no effect on the formation of the pigments. However, reduction in the concentration of the MS inorganic salts in the basal medium resulted in less pigment formation. One-tenth strengh of the MS inorganic salts in the basal medium appears to be optimal among the concentrations of salts tested (Table 1). Further improvement of the basal culture medium involved the complete omission of sucrose and the organic supplements (Nitsch & Nitsch 1965). In summary, the most effective culture medium to minimize the dark coloration of the micro-cutting cultures consisted of the one-tenth strength of the inorganic salts (Murashige & Skoog 1962) at pH 5.8 solidified with agar (0.8% w/v). This culture medium, designated the MIN medium was also apparently adequate for the growth and further development of the micro-cutting cultures and was used in the rest of the investigations described here.

Addition of GA_3 at 10 mg l^{-1} to the MIN medium was by far the most effective way to obtain whole plantlets from micro-cuttings excised from *H. speciosa* grown outdoors in late autumn and winter months (Table 2). On this medium, internodal elongation and shoot development occurred in about 60-70% of the explants. After 2 months in culture, the micro-cuttings grew up to 3 cm tall with up to 4 visible nodes. For root initiation, these *in vitro* shoot cultures were transferred to the MIN medium containing 0.1 mg l^{-1} IBA. Within 2 to 3 weeks, roots were formed in over 90% of the cultures. In contrast, about 50%, of those obtained from the same plants during the spring growth period had the capacity to form roots and then commence internodal elongation resulting in the development of entire plantlets in the absence of any plant growth regulators. Addition of IBA (0.1 mg l^{-1}) was only marginally more stimulatory. However, about 80% of the springtime micro-

Table 1. Effect of salt concentration on the amount of brown/black pigmented exudate produced by micro-cuttings.

Media	Exudate
MS salts + 3% Sucrose + Organics	++++
1/2 MS salts + 3% Sucrose + Organics	++++
1/5 MS salts + 3% Sucrose + Organics	+++
1/10 MS salts + 3% Sucrose + Organics	++
1/20 MS salts + 3% Sucrose + Organics	++
1/10 MS salts (No Sucrose or Organics)	+

All media contained GA_3 (10 mg l^{-1})

Table 2. Effect of GA_3 on the percentage of mico-cuttings that develop shoots.

Conc GA_3 (mg l^{-1})	Expt 1	Expt 2	Mean
1	30%	20%	25%
5	50%	40%	45%
10	60%	70%	65%
20	60%	60%	60%

n = 10 shoots per Expt

cuttings developed into plantlets when cultured on the MIN medium, first containing GA_3 (10 mg l^{-1}) and then IBA (0.1 mg l^{-1}) in the same way as observed with the micro-cuttings from the winter months.

Having established axenic *in vitro H. speciosa* plantlets, we proceeded to examine an effective way to micropropagate these materials. Single nodal explants from the *in vitro* plantlets were cultured on the MIN medium alone or supplemented with 0.1 mg l^{-1} IBA. Addition of IBA sped up root development in the nodal explants removed from the plantlets recently established in culture from the micro-cuttings. Nodes from plantlets which had undergone several culture cycles required no exogenous plant growth regulators in the MIN medium to develop into plantlets. Root development usually took place 2 to 4 weeks from the start of nodal explant culture and before axillary shoot/bud elongation in the nodes. After 8 weeks in culture, the leafy shoots arising from the axillary buds were between 1 and 3 cm long with up to 4 visible nodes. The single nodal explants excised from these shoots can be used for another cycle of multiplication. This process of micropropagation may be repeated and continued throughout the year. It was also found that the *in vitro* plantlets could be maintained on the MIN medium without subculture for at least 5 months.

The plantlets from the nodal explants were successfully established *in vivo* when removed from tissue culture vessels and placed in pots containing vermiculite in a growth room at 25°C day 15°C night and 16 h photoperiod.

DISCUSSION

Shoot tips or micro-cuttings containing meristems are generally thought to be the explants most amenable for *in vitro* plant multiplication and a cytokinin is required in many cases (Binding & Krumbiegel-Schroeren 1984). Our results with *Hebe speciosa* show that GA_3 and not cytokinin (BA or kinetin) stimulated a high frequency of the micro-cuttings to undergo shoot elongation in culture. This is not entirely surprising as GA_3 is known to promote internodal growth in many plants (Bidwell 1974). However, the use of GA_3 in micropropagation has not been a common practice.

The response of the micro-cuttings on culture media without added plant growth regulators was found to be subject to seasonal influences. This parallels the difficulties commonly encountered with conventional propagation practices. We suggest that the difficulties might arise from the possible endogenous changes in the hormonal metabolism and/or sensitivity of the stock plants kept outdoors during different seasons.

An important observation here was that the media components could interact with the micro-cutting explants in producing the dark/brown colouration of the media. This enabled us to develop a low salt, vitamin and sucrose-free medium which is apparently adequate for shoot development and growth.

Once axenic plantlet cultures of *H. speciosa* have been successfully established, then for all the subsequent *in vitro* multiplication cycles single nodal explants were found to develop whole plants without added plant growth regulators although IBA appeared to speed up the multiplication process.

Several advantages are apparent with the micropropagation process described here over the conventional propagation methods. Firstly, stock plants may be maintained in culture vessels and thus a minimal space is required. Secondly, the seasonal influences on plant multiplication can be circumvented as the *in vitro* plantlets kept under constant culture conditions can be used as the source of explants throughout the year. Thirdly, a high multiplication rate is possible with a single node having the potential to produce over 2000

plants over a one year period. Fourthly, the procedures outlined in this paper could easily be adapted for the multiplication of many other endangered plant species.

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